Eur J Vasc Endovasc Surg **30**, 315–318 (2005)
doi:10.1016/j.ejvs.2005.05.002, available online at http://www.sciencedirect.com on SCIENCE DIRECT®

Hyperhomocysteinaemia as a Risk Factor for Deep-vein Thrombosis

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Objective. *Several studies have shown a relation between hyperhomocysteinaemia and vascular disease. To assess the risk of deep-vein thrombosis (DVT) associated with hyperhomocysteinaemia, we studied plasma homocysteine levels in patients with deep-vein thrombosis and in normal control subjects.*
Materials and methods. *We measured plasma homocysteine levels in 48 patients with deep-vein thrombosis and in 33 healthy controls matched to the patients according to age and sex. Plasma homocysteine levels were measured with high performance liquid chromatography and fluorescence detection. Hyperhomocysteinaemia was defined as a plasma homocysteine level about 15 μmol/L in both groups. The diagnosis of all patients with deep-vein thrombosis (*n*=48) was verified by Doppler ultrasonography.*
Results. *Plasma homocysteine levels were found to be increased in the deep-vein thrombosis group compared the control group (*p*<0.001, t-test). The mean plasma homocysteine level in the patients was 17.1 SD 5.13 μmol/L (range 6.4–31.3), and that in the controls was 9.0 SD 1.27 μmol/L (range 6.0–11.5).*
The association between elevated homocysteine levels and venous thrombosis was stronger among men than among women.
Conclusions. *The increased plasma homocysteine levels we have observed may have a causative role in the development of deep-vein thrombosis.*

Keywords: Deep-vein thrombosis; Hyperhomocysteinaemia; Risk factor.

Introduction

Hyperhomocysteinaemia (tHcy) is epidemiologically associated with an increased risk of myocardial infarction, stroke and peripheral arterial thrombosis as well as venous thromboembolism.[1–5] Hyperhomocysteinaemia is a disorder of methionine metabolism. Folic acid is the major dietary determinant of the plasma homocysteine (Hcy) concentration, as shown in a Dutch population study.[6] Folate deficiency may be caused by suboptimal folate intake, by increased physiologic demands and through altered metabolism of iatrogenic origin. In such deficiency states, folic acid supplementation will quickly and efficiently normalise plasma Hcy.[5]

Normal human plasma contains total concentrations of Hcy and its derivative disulfides close to 10 μmol/L, although there is some variation due to genetic factors, age, sex, menopausal status, and other physiological and lifestyle variables.[7,8] Hyperhomocysteinaemia is the presence of an abnormally elevated concentration of plasma or serum the totality of Hcy. Some authors have defined the quantitative degree of tHcy by use of terms such as 'moderate, intermediate, and severe' or 'mild, intermediate, and severe'.[8,9] Several studies have shown a relation between mild tHcy and vascular disease.[10–12] Homocystinuria was diagnosed by measuring Hcy in urine by using qualitative methods or amino acid analysis that detected Hcy.[8] In classic homocystinuria, half the vascular complications are of venous origin,[13] but until recently it has been unclear whether mild tHcy is also a risk factor for venous thrombosis.[11,14] In a case-control study, Falcon *et al.* found that tHcy was a risk factor for thrombosis in people younger than 40 years of age.[15,16] Since, tHcy also appears to be common, we examined the risk of thrombosis in persons with deep-vein thrombosis (DVT).

Materials and Methods

Patients were selected from the files of our clinic. Of

This Clinical Chemistry article was present at the 54th Annual Meeting, Orange Country Convention Center, Orlando, FL, USA, July 28–August, 2002.
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1078–5884/000315+04 $35.00/0

the 181 patients approached, 106 participated. Twenty-five of 106 patients were excluded because of positive tests for thrombophilia.

We recruited the control group through a general practice in our hospital. We invited 122 people aged 34–71 from this practice to take part in a health survey of risk factors for cardiovascular disease. Forty-seven people agreed to participate and the first 33 healthy persons formed the control group. All patients were tested for thrombophilia. We tested for antithrombin III, protein C, protein S, activated protein C resistance, lupus anticoagulant, prothrombin time, activated partial thromboplastin time, bleeding time, and platelet count in this group. We excluded volunteers from the control group when any test was positive.

Forty-eight patients (14 women, 34 men) presenting with DVT between 1999 and 2001 were included in the study. The diagnosis was confirmed by duplex ultrasonography in all patients. Of these patients 33 presented with an acute DVT while the remainder ($n=15$) had recurrent DVT following an earlier episode. All patients ($n=15$) with a history of recurrent venous thrombosis were managed by oral anticoagulant therapy and are registered at our clinic. The patients with recurrent DVT had had two or more episodes of venous thrombosis. From this group we excluded those with thrombophilia (protein C, protein S, and antithrombin III deficiency), cardiorespiratory and rheumatic diseases requiring extensive medical therapy, known malignant tumours, pregnancy, and other contraindications for oral anticoagulation therapy (severe arterial hypertension, severe hepatic failure, recent peptic ulcer disease, and so forth). We obtained a short medical history from all patients by interview and all controls by questionnaire.

We obtained blood samples from the antecubital vein at 08.00 h in 5 mL vacutainer tubes and 4–5 mL EDTA vacuum glass tubes for determination of the Hcy. The EDTA-samples for Hcy measurement were immediately placed on ice and centrifuged at 3500*g* for 5 min within 15 min of venesection. The plasma was separated and stored at $-20\,^{\circ}C$ until analysis (approximately 3 months).

The total Hcy concentration was measured by an isocratic system with high performance liquid chromatography (HPLC) pump (flow rate 1.7 mL/min), injector (injection volume 20 μL, analytical run time 4–5 min) and fluorescence detector (Ex: 385 nm, Em: 515 nm) (HP 1100). Hyperhomocysteinaemia was defined as a plasma Hcy level above 15 μmol/L in both groups.

In order to evaluate the possibility of a dose-response relation, we stratified the patients and controls according to their Hcy concentrations and calculated odds ratios for thrombosis in the patients at the higher levels as compared with those at the lowest level. In addition, we investigated the association between tHcy and venous thrombosis in men and women. The local ethics committee approved the study protocol, and all participants gave their informed consent. This study was completed as retrospective case control study with a limited number of individuals.

Statistical analysis

Data are expressed as mean values with standard deviations. Receiver operating characteristic (ROC) analysis was used calculate the sensitivity and specificity of parameters.

An independent sample *t*-test was used and two-tailed *p* value less than 0.05 was considered statistically significant.

We calculated matched odds ratio to estimate the relative risk of thrombosis for homocysteine values above a given point, with the matching factor taken into account. The 95% confidence intervals were calculated from a conditional logistic-regression algorithm by the maximum-likelihood method. We also calculated the cut-off point for Hcy, which was ≥ 11.0 μmol/L.

Results

The mean age of the patients with DVT ($n=48$; 14 women, 34 men) was 52 years (21–75 years). Thirty-three patients (9 women, 24 men) recruited contemporaneously formed the healthy control group. The mean age of the patients was 55 years (34–71 years).

The mean plasma Hcy level in the patients was 17.1 SD 5.13 μmol/L (range 6.4–31.3), and that in the controls was 9.0 SD 1.27 μmol/L (range 6.0–11.5) (Table 1). The ratio Hcy levels in male to female subjects among both the patients and the controls was 1.2:1. The Hcy concentrations of individual case patients and controls are shown as a scattergram in Fig. 1. The Hcy concentrations between the patients with DVT and the control group were significantly different ($p<0.001$). The mean Hcy levels for men and women in the patient group were 18.12 SD 5.40 μmol/L and 14.54 SD 3.35 μmol/L, respectively. The higher plasma level of tHcy in men than in women was present at all ages ($p<0.01$ for the comparison between the sexes).

The 73rd percentile of the Hcy levels in the control group was 6–9.9 μmol/L. The cut-off point was

Table 1. Base-line characteristics of the plasma homocysteine values in patients with deep-vein thrombosis and in controls

	N	Range	Minimum	Maximum	Mean	Standard deviation
Control	33	5.5	6.0	11.5	9.0	1.27
Patients	48	24.9	6.4	31.3	17.1	5.13

Patients and control subjects with other thrombophilias have been excluded from this study.

defined as a level of $\geq$11.0 µmol/L based on the distribution between the patients with DVT and the control subjects. These cut-off points are comparable to the reference values obtained from the patients with 17.1 µmol/L and the controls with 9.0 µmol/L for Hcy concentration. The 45 patients (93.7%) with DVT had levels exceeding the cut-off point of 11.0 µmol/L, *versus* two patients (6.1%) in the control group. The results of calculation using other cut-off values is shown in Table 2. When the cut-off was set at the 90th percentile, the matched odds ratio for the risk of venous thrombosis was 2.32 (Table 3).

Discussion

Our study shows that tHcy is a risk factor for DVT in the general population. Our results also suggest that the association between mild tHcy and DVT is similar in degree to that reported for tHcy in published literature.[16] We found an increased Hcy concentration in patients compared to controls. When we analysed men and women separately, we found a difference in the risk of thrombosis associated with tHcy, and women had lower levels of plasma Hcy than men as discussed by Lussier-Cacan.[17]

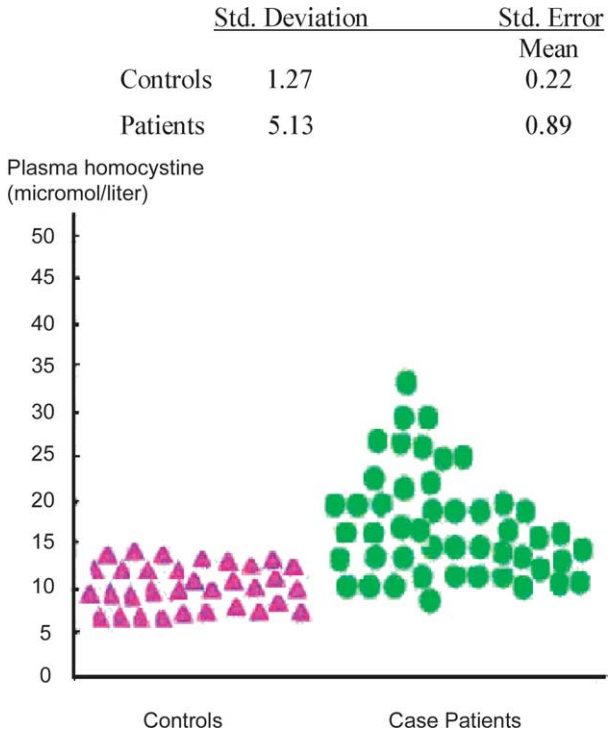

Fig. 1. Plasma homocysteine levels in 33 controls and in 48 patients with deep-vein thrombosis.

Hyperhomocysteinaemia is a common risk factor for recurrent venous thrombosis.[18] Patients with homocysteinemia have more frequent thrombotic complications such as DVT.[13]

Many hypotheses have been proposed to explain how tHcy may lead to venous thrombosis. One hypothesis is that Hcy has a toxic effect on the vascular endothelium and on the clotting cascade.[10,11,16]

The normal fasting range of plasma homocysteinemia is 5–15 µmol/L.[19] Hyperhomocysteinaemia is considered to be present when plasma Hcy levels exceed 15 µmol/L.[9] Mild increases in plasma Hcy levels (>16 µmol/L) have been associated with the presence of vascular disease. A plasma Hcy concentration of more than 22 µmol/L increased the matched odds ratio for DVT to 4.0.[12]

We also found an increasing odds ratio for the risk of DVT with an increasing Hcy concentration. This finding implies that there is no definite sharp cut-off point. For Hcy this would be at a level of 11.0 µmol/L for the fasting value. Determination of odds ratio is important because of the high prevalence of tHcy in the general population.

The effect of tHcy was independent of other well-established risk factors for thrombosis, including protein C, protein S, and antithrombin III deficiencies and activated protein C resistance[20] since, we sought

Table 2. Results of ROC curve analysis

	Control	Case patients
Cut-off: 73rd percentile (9.9 µmol/L)		
Above cut-off	9	47
Below cut-off	24	1
Cut-off: 85th percentile (10.3 µmol/L)		
Above cut-off	5	47
Below cut-off	28	1
Cut-off: 90th percentile (11.0 µmol/L)		
Above cut-off	2	45
Below cut-off	31	3
Cut-off: 97.5th percentile (11.5 µmol/L)		
Above cut-off	1	42
Below cut-off	32	6

Different cut-off points for homocysteine levels and the effect on the proportions of controls and patients falling below the resulting level.

Table 3. Odds ratios for thrombosis associated with hyperhomocysteinaemia

	Odds ratio
80th percentile (12.8 μmol/L)	1.98
90th percentile (15.1 μmol/L)	2.32
95th percentile (18.2 μmol/L)	2.57

and excluded patients with these thrombophilias from our study.

Ebbesen *et al*. revealed that the whole blood coagulation profile was influenced by high plasma Hcy by (1) prolonging the initiation phase, (2) increasing the velocity of the coagulation propagation and (3) increasing the maximum clot firmness. These changes in whole blood coagulation profile may contribute to the increased risk of thrombosis in hyperhomocysteinemic individuals.[5] Elevated Hcy levels may result from low levels of folic acid, vitamin B_6, or vitamin B_{12}. It remains unclear whether tHcy of different causes entails the same risk of thrombosis. Nevertheless, it is well known that vitamin supplementation lowers Hcy concentrations and reverses endothelial dysfunction in almost all subjects with tHcy, regardless of the underlying cause.[16,21]

We conclude that mild tHcy is a risk factor for DVT in the general population.

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Accepted 3 May 2005
Available online 15 June 2005